AMERICAN JOURNAL OF EPIDEMIOLOGY Vol. 96, No. 2
 Printed in U.S.A.

VIROLOGIC STUDIES OF ACUTE RESPIRATORY DISEASE IN YOUNG ADULTS

V. CORONAVIRUS 229E INFECTIONS DURING SIX YEARS OF SURVEILLANCE

DOROTHY HAMRE AND MARC BEEM[1]

(Received for publication November 24, 1971)

Hamre, D. and M. Beem (Dept. Pediatrics, Univ. of Chicago, Chicago, Ill. 60637). Virologic studies of acute respiratory disease in young adults. V. Coronavirus 229E infections during six years of surveillance. *Am J Epidemiol* 96: 94–106, 1972.—In a surveillance study of acute respiratory disease in medical students that spanned six consecutive seasons between 1961 and 1968 and encompassed 937 student years of observation, infection with coronavirus 229E was identified by virus isolation and serologic studies. Virus isolation identified 12 infections, 8 in one season, 4 in another. Complement fixing (CF) antibody titer rises identified 133 infections that occurred in all six seasons of surveillance, involving from 15 to 35% of students in three seasons of "high" prevalence, and 1 to 5% in intervening seasons of "low" prevalence. Infection occurred in a winter-spring seasonal pattern and was associated with acute respiratory illness that was not clinically distinctive. Neutralizing antibody to 229E was commonly present in the sera of the students. The level of this did not appear to influence the occurrence of, or likelihood of illness with, reinfection as judged by CF seroconversion; however, the frequency of significant rise in neutralizing antibody titer with reinfection was inversely related to pre-infection levels of this antibody. Infection with other common respiratory viruses did not stimulate significant CF or neutralizing antibody titer rises to 229E.

coronaviruses; respiratory tract infections; serology; viruses

INTRODUCTION

The isolation in cell culture and characterization of a new respiratory virus designated "229E" has previously been reported from this laboratory (1). It has subsequently been shown that this ether and acid labile RNA virus shares morphologic and biophysical characteristics with other human respiratory viruses that can be isolated only in organ culture of human respiratory epithelium (2, 3) and that these human respiratory viruses, in turn, are morphologically similar to avian infectious bronchitis virus (IBV) and mouse hepatitis virus (MHV) (3–5). The term "coronavi-

Abbreviations: CF, complement fixing; CPE, cytopathic changes; HDF, human diploid fibroblasts; HI, hemagglutination inhibition; HK, human kidney; IBV, infectious bronchitis virus; MHV, mouse hepatitis virus; MK, monkey kidney; RS, respiratory syncytial.

[1] From the Departments of Medicine and Pediatrics, University of Chicago, 950 E. 59th St., Chicago, Illinois 60637.

This investigation was supported by Public Health Service Grant NIH-5-RO1-AI-03292, contract number PH-43-63-564 from the Vaccine Development Branch, National Institutes of Allergy and Infectious Diseases, General Research Support Grant PHS FR-5367, and the Children's Research Foundation, Western Springs, Ill.

The authors gratefully acknowledge the technical assistance of Evelyn Saxon.

rus" has been proposed for this group of viruses (6).

Definition of the antigenic composition of the human coronaviruses has been limited by the small number of isolations so far reported (14 in tissue culture, nine in organ culture) and the inability to adapt most of the organ culture strains to more practical laboratory systems. At least two immunotypes have so far been distinguished: one consisting of 229E and all of the other strains derived from primary isolation in cell culture, and the other of organ culture strains OC 38 and 43 (7). The number of additional immunotypes represented by the remaining organ culture strains is as yet uncertain.

That human respiratory coronaviruses have the potential to be respiratory pathogens has been demonstrated in artificial challenge studies: common colds have developed in significant numbers of volunteers inoculated with coronavirus strains B814 and 229E (2, 8). However, little is known about the extent and significance of infection with these viruses in naturally occurring acute respiratory illnesses. The relatively complex methodology of organ culture systems limits the applicability of this technique to etiologic studies, and, although the primary isolation of 229E-like strains can be accomplished in cell culture, this is usually quite difficult to do. Even so, the isolation of multiple 229E-like strains from adults with acute respiratory illness has previously been reported on two occasions (1, 9), and a third is described below.

The application of serologic techniques to the question of prevalence and significance of human respiratory coronavirus infections has been circumscribed by incomplete knowledge of the serologic interrelations of the group and the restriction of laboratory methods suitable for such studies to tissue culture strain 229E and organ culture strain OC 38/43. Antibody prevalence surveys indicate human infection with these and/or related coronaviruses may be quite common (8, 10, 11), and serologic studies have suggested an etiologic role for both 229E (9, 10) and OC 38/43 (9, 12) in acute respiratory illness that was not selected on the basis of severity. However, in a study of acute lower respiratory disease of infants and children that necessitated hospitalization, there was no complement fixing (CF) antibody evidence that infections with these viruses played a significant role (11).

The following report of serologic and epidemiologic studies of 229E virus is based upon observations made in the course of a continuous surveillance of respiratory disease among medical students at the University of Chicago during a six-year period between 1961 and 1968. It includes observations on 1) CF and neutralizing antibody responses to 229E of eight previously reported (1) and four additional individuals from whom 229E virus was isolated, 2) the persistence of neutralizing and CF antibody in 12 other individuals in whom infection with this virus was diagnosed serologically, 3) the occurrence of neutralizing and/or CF antibody responses to 229E virus in the course of infection with other respiratory viruses, 4) the overall occurrence during the six years of respiratory disease surveillance of CF antibody rises to 229E, 5) the relationship between neutralizing and CF antibody rises to 229E in 189 of the students under surveillance during the 1966–1967 season, and 6) the relation between 229E virus infection and acute respiratory illness.

Materials and methods

The methods employed for surveillance of the medical students and isolation and identification of respiratory viruses have been reported in detail previously (13, 14). They were briefly as follows:

Medical student surveillance. Medical students participated in the program on a voluntary basis. Only students in the first two years of school were included during 1961 through 1963. After that students in their third and fourth years, as well as a few who had gone on to internship and residency were included. Students reported to

the laboratory at the first sign of an acute illness and secretions from the nose and throat were obtained on cotton swabs which were subsequently placed in collecting medium. The first of two "illness" blood specimens were obtained at the time of the original visit and the second two to five weeks later. At this time a brief form describing the symptoms and duration of illness was completed. Additional blood specimens and cultures were obtained from all participants at six-week intervals on a routine basis.

Virus isolation. Over the several years of the study there were minor variations in the media and cell cultures employed for virus isolation. Throughout the study all specimens were inoculated into cell cultures of rhesus monkey kidney (MK) and HEp-2. Human kidney (HK) cell cultures were also employed routinely in 1961–1962. After this time, human diploid fibroblasts (HDF), WI-38, or HEL-1 (a University of Chicago strain), were routinely employed and HK was only used periodically.

Cell cultures inoculated with specimens, and an appropriate number of controls, were incubated at 36 C (MK, HEp-2) or 33 C (HK, HDF), stationary (MK, HEp-2), or rolling (HK, HDF) and observed at regular intervals over a 20-day period for the development of cytopathic changes (CPE); MK cultures were also tested for hemadsorption.

Virus isolates were identified by neutralization of infectivity or hemadsorption inhibition employing appropriate antisera.

CF test. Bottles containing confluent HDF monolayers were drained of medium and inoculated with enough 229E virus to provide about one plaque forming unit per cell. After a one hour absorption at 33 C, enough maintenance medium (Eagle's Minimal Essential Medium with 1 per cent fetal calf serum) was added to cover the monolayer and the bottles incubated until the first appearance of CPE could be detected (about 36 to 48 hours later). The bottles were then frozen and thawed three times and the debris removed by centrifugation. The supernate was divided into small aliquots and refrozen for use as CF antigen.

The CF test was performed in microtiter plates essentially using the method of Sever (15). Antigen was titrated by the checkerboard method, employing acute and convalescent sera of a student from whom the virus was isolated in 1961. Two units of antigen were employed in the test, along with two exact units of complement which had been titrated in the presence of antigen. After overnight fixation at 4 C, the hemolytic system was added and the plates incubated at 37 C for 30 minutes. Readings were made after the cells had settled in the refrigerator for three to four hours. Titers represent the highest dilution showing 3+ to 4+ fixation.

Neutralization tests. Twofold dilutions of heat inactivated serum (56 C for 30 minutes) were made in beef heart infusion broth and mixed with an equal volume of virus diluted to give 5–50 $TCID_{50}$ of virus per 0.2 ml of virus-serum mixture. After incubation at room temperature for two hours, three tubes of HDF were inoculated with 0.2 ml of each virus-serum mixture. Appropriate virus controls and titrations were included in each test to provide a concurrent determination of the actual $TCID_{50}$ of virus used in the test. Final readings were made after three to five days of incubation on roller drums at 33 C, when virus control tubes showed 3+ to 4+ CPE. Tubes showing any degree of CPE were considered positive and endpoint titers represent the highest dilution of serum neutralizing the virus dose (indicated in parentheses in table 1) in at least two of the three inoculated cell cultures.

Serologic observations on students from whom 229E was isolated

Twelve 229E infections were identified by virus isolation in the six-year surveillance period and the serum neutralizing and CF

antibody responses to 229E that occurred following these infections are presented in table 1.

Neutralizing antibody was present in the pre-infection sera of eight of 12 students at titers of 1:2 to 1:16. Of the four students "without" pre-infection antibody, three had titers <1:8 and one was <1:4. Post-infection, eight of the 12 developed fourfold rises in titer, with 1:64 the peak titer observed. CF antibody was absent (<1:4) from all pre-infection sera. Post-infection sera showed fourfold or better rises in seven of the 12 and an additional student had a two-fold titer rise.

Thus, in this small experience, two thirds of individuals with virus-shedding infections developed significant rises in either CF or neutralizing serum antibody titer. The majority of the negative serologic responses of infection were contributed by three students who failed to develop either neutralizing or CF antibody titer rises and were alike in the additional respect that all were "without" pre-infection neutralizing antibody. It was not possible to reconfirm these isolations because material was not available for reisolation attempts, but it was determined that the strains of virus isolated from these students were antigenically similar to the prototype to the extent that they and the prototype strain were neutralized with equal efficiency by 229E hyperimmune guinea pig serum. It was also determined that these students failed to develop a neutralizing antibody response to their own as well as the prototype strain of 229E.

TABLE 1

Serum CF and neutralizing antibody response to 229E virus: 12 students from whom this virus was isolated

Student identification	Antibody titer to 229E*			
	Neutralizing		Complement fixing	
	Pre	Post	Pre	Post
220	*8*	***32*** (5)†	*<4*	*16*
227	*<8*	***32*** (5)	*<2*	*4*
229	*2*	***32*** (30)	*<4*	*8*
241	*4*	***32*** (50)	<4	<4
243	*8*	***32*** (50)	*<4*	*16*
276	*4*	*≥**32*** (50)	*<4*	*8*
297	<8	8 (5)	<4	<4
299	16	32 (30)	*<4*	*8*
0661‡	<8	<8 (30)	<4	<4
0744‡	*16*	*64* (30)	*<4*	*16*
0765‡	*8*	***32*** (60)	*<4*	*4*
0312‡	<4	<4 (60)	<4	<4

* Reciprocal of initial serum dilution; significant rises italicized.

† $TCID_{50}$ of virus used in test.

‡ These 229E virus isolations not previously reported.

PERSISTENCE OF CF AND NEUTRALIZING ANTIBODY

In 12 other medical students, 229E infection was identified by CF and neutralizing antibody seroconversion early in the course of their participation in this surveillance study. Serial serum specimens were available from them that extended over a two- to four-year period. The persistence of antibody observed in these students is summarized in table 2. Considering neutralizing antibody first, three of the 12 "lacked" antibody to 229E virus in the pre-rise serum while the remainder had titers ranging from 1:4 to 1:16. Peak titers ranged from 1:16 to ≥1:128 and 22 to 28 months later titers were still significantly higher than pre-rise levels in seven of the 12 students. In contrast, CF antibody titers were <1:4 in the initial sera of all 12 students, rose to peak values of 1:4 to ≥1:16 and in all cases returned to <1:4 within one to nine months following peak values. The persistence of neutralizing and evanescence of CF antibody to 229E implied by the common occurrence of neutralizing but not CF antibody in pre-infection sera of students, is demonstrated in these data. Also, the presence of neutralizing antibody in the pre-infection sera of nine of these 12 students with seroconversion to 229E, as well as eight of the 12 from whom virus was iso-

TABLE 2

*Persistence of CF and neutralizing antibody following 229E virus infection**

Student identification	Antibody titer to 229E†							
	Neutralizing				Complement fixing			
	Pre-rise	Peak	Final	Time interval—peak to final	Pre-rise	Peak	Final	Time interval—peak to <4
0237	<4	128	>32	3 yrs 2 mos	<4	≥16	<4	6 mos
0531	<4	16	8	3 yrs	<4	8	<4	8 mos
0503	<8	32	16	3 yrs 2 mos	<4	8	<4	2 mos
0459	16	64	32	1 yr 10 mos	<4	8	<4	3 mos
0516	8	≥128	≥64	3 yrs 5 mos	<4	8	<4	6 wks
0535	16	≥128	32	2 yrs 2 mos	<4	16	<4	3 mos
0532	4	32	16	3 yrs 3 mos	<4	16	<4	9 mos
0505	8	≥128	8	3 yrs 11 mos	<4	16	<4	8 mos
0515	16	≥128	≥64	3 yrs	<4	4	<4	6 wks
0453	8	≥64	16	2 yrs 2 mos	<4	8	<4	6 wks
0486	4	16	16	4 yrs	<4	4	<4	4 wks
0498	8	32	16	2 yrs 9 mos	<4	8	<4	5 mos

* Infection identified by 4-fold or greater rise in neutralizing and 2-fold or greater rise in CF antibody titer.

† Reciprocal of initial serum dilution.

lated, underscores the natural occurrence of reinfection with this virus.

SPECIFICITY OF SEROLOGIC RESPONSE TO 229E VIRUS

Information concerning the specificity of the serologic responses to 229E virus was sought by examining pre- and post-infection sera of students with virus shedding respiratory infections caused by other viruses. CF and neutralizing antibody responses to 229E virus were determined following infection with rhinovirus (82 students), herpesvirus (12 students), respiratory syncytial (RS) virus (16 students), influenza virus (A_2—two students, B—10 students), parainfluenza virus (type 1—four students, type 2—five students, type 3—six students), and adenovirus type 1 (one student). Four increases of 229E CF antibody titer were observed: two followed rhinovirus infections and one each infection with herpesvirus and adenovirus. With two of the CF rises (herpesvirus—one, rhinovirus —one), there were concomitant increases in the titer of neutralizing antibody to 229E. All rises took place during time periods of known 229E prevalence. Since these CF titer rises were few in number, accompanied in two of the four instances by rises in neutralizing antibody titer, and occurred during times when concomitant infection with 229E was possible, these data are interpreted as showing little, if any, evidence of heterologous responses to 229E in the course of infection with the indicated respiratory viruses.

CF ANTIBODY SEROLOGIC SURVEY— ALL SURVEILLANCE YEARS

Because the cytopathic changes of 229E in human diploid cell cultures are difficult to detect, it seemed probable that a serologic test would better estimate the true incidence of infection in the medical student group. For this, CF tests were performed on all sera collected between November 1961 and May 1968, including both "routine" specimens collected at six-week intervals and acute and convalescent "illness" specimens. In doing the CF tests, it was found that almost all sera were completely nega-

TABLE 3

229E virus isolations and antibody rises among medical students by surveillance year

No(s). of students	Surveillance year						Total
	1961–62	1962–63	1963–64	1964–65	1965–66	1966–67	
Under surveillance	110	116	137	182	201	191	937
With 229E virus isolation	8					4	12
With CF antibody rises*	34 (31%)	6 (5%)	21 (15%)	1 (1%)	5 (3%)	66 (35%)	133 (15%)
With neutralizing antibody rises†	20	0	14	1	2	29	66

* CF rises included seroconversions (<4 to 4) confirmed by retesting as well as instances of fourfold or greater rises (see text).

†Neutralization tests performed only on students with CF rises.

tive for complement fixation at the initial 1:4 dilution. When, in a series of specimens from one student, a positive reaction was found at but not beyond this 1:4 dilution, the immediately following sera also showed complement fixation that was at the same level or progressively declined through 2+ or 1+ and then became, and remained, completely negative. It was decided, therefore, that CF titer changes from <1:4 to ⩾1:4 that could be confirmed on repeat testing were serologically significant.

CF antibody titer rises and 229E isolations found in the entire group of students together with neutralizing antibody titer rises of students with CF seroconversions are presented in table 3. In the overall experience, which encompassed 937 "student years" of observation, 133 students with CF antibody titer rises were identified. One or more students developed rises in each of the six seasons and the frequency of rises in any one season appeared to fall into one of two patterns, "high" or "low". Thus, there were three "high" seasons with 15 to 35 per cent of students showing rises (1961–1962—31 per cent, 1963–1964—15 per cent, 1966–1967—35 per cent) and three "low" seasons with 1 to 5 per cent showing rises (1962–1963—5 per cent, 1964–1965—1 per cent, 1965–1966—3 per cent). The high frequency seasons did not occur consecutively but were separated by one or two low frequency seasons.

High and low frequency seasons also differed in respect to the portion of the observed CF rises that were "2×" (<1:4 to 1:4) and "⩾4×" (<1:4 to ⩾1:8). In high frequency seasons, both 2× and ⩾4× rises occurred, with the latter comprising 76, 48 and 74 per cent of CF rises in the 1961–1962, 1963–1964 and 1966–1967 seasons, respectively, and 64 per cent (85/133) of all CF rises observed. In low frequency seasons, only 2× rises were seen and in this respect low frequency seasons differed significantly from high frequency seasons ($p < .001$).

NEUTRALIZING ANTIBODY RISES TO 229E

Although CF cross-reactions between 229E and other viruses have not been described, the specificity of this test is uncertain. Therefore, neutralization tests were also done on the sera of students with CF rises. Of the 133 students with CF rises, 66 (50 per cent) had concomitant neutralizing antibody rises. The portion of students with neutralizing antibody rises showed a direct relation to the extent of CF antibody rise (table 4), being 35 per cent of 48 students with 2× CF rises, 45 per cent for 53 students with 4× rises and 78 per cent of 32 students with ⩾8× rises. Among students with 2× CF rises, concomitant neutralizing antibody rises occurred less often in low than in high frequency years (3/12 vs 14/36) but

TABLE 4

Neutralizing antibody titer rises in students with CF antibody titer rises according to extent of CF rise*

Season	Fold increase in CF titer					Totals
	<2×†	2×	4×	8×	≥16	
1961–62	ND‡	4/8§	9/18	3/4	4/4	20/34
1962–63	ND	0/6				0/6
1963–64	ND	4/11	6/6	4/4		14/21
1964–65	ND	1/1				1/1
1965–66	ND	2/5				2/5
1966–67	(4/123)¶	6/17	9/29	11/15	3/5	29/66
Totals		17/48	24/53	18/23	7/9	66/133

* ≥4-fold increases in titer.

† <4 − 4 = 2×; <4 − 8 = 4×, etc.

‡ Not done.

§ No. with neutralizing antibody titer rise/no. with this extent of CF antibody titer rise.

¶ Not included in totals.

this difference did not appear to be significant ($p = .5$). One or more neutralizing antibody titer rises occurred in all except one season, 1962–1963.

COMPARISON OF CF AND NEUTRALIZING ANTIBODY SEROCONVERSION RATES AND PREVALENCE IN 1966–1967

To further compare the CF and neutralization tests, neutralizing antibody titers were determined in the November, January and May sera of all but two of the students under surveillance in the 1966–1967 season (table 5). Of the 189 students with sera surveyed by both tests, significant rises in neutralizing antibody titer occurred in 33 (17 per cent) while 66 (35 per cent) developed CF rises. Four students showed seroconversion by neutralization test only and 37 by CF test only. Thus, CF seroconversion to 229E occurred twice as often as neutralizing antibody seroconversion and the CF test alone identified 66 (94 per cent) of the 70 students with seroconversion to 229E that were found when both tests were used. In the November sera of these students, CF antibody titers were uniformly <1:4, while 169 (89 per cent) of the 189 students had neutralizing antibody at titers ≥1:4.

RELATION OF PRE-INFECTION SERUM NEUTRALIZING ANTIBODY LEVEL TO CF AND NEUTRALIZING ANTIBODY SEROCONVERSION

The serologic findings in the students from whom 229E virus was isolated indicated that the immune state characterized by serum neutralizing antibody to this virus did not preclude natural reinfection. Other serologic evidence supported this. In the 1966–1967 season, pre-infection neutralizing antibody titers ≥1:4 were found in 62 (94 per cent) of 66 students with CF seroconversion and in 28 (85 per cent) of 33 students with neutralizing antibody seroconversion; in the other five seasons of surveillance, of 67 students with CF seroconversion, 46 (69 per cent) had pre-rise neutralizing antibody titers ≥1:8.

To determine how reinfection was related to the level of neutralizing antibody, neutralizing and CF antibody seroconversion rates were determined for the 189 students of the 1966–1967 season according to the level of neutralizing antibody found in their November 1966 serum specimen (table 6). A distinctly different relationship was found between the preinfection neutralizing antibody level and the frequency of seroconversion as determined by the two tests. The frequency of neutralizing antibody seroconversion was inversely related to the

TABLE 5

Serum neutralizing and CF antibody rises to 229E among 189 students in the 1966–67 surveillance year

Neutralizing antibody titer rise*	Complement fixing antibody titer rise†		Total with indicated neutralizing antibody response
	Yes	No	
Yes	29	4	33
No	37	119	156
Total with indicated CF antibody response	66	123	189

* ≥4-fold rise in titer between November 1966 and May 1967.

† Titer increase from <1:4 to ≥1:4.

TABLE 6

Neutralizing and CF antibody seroconversions to 229E according to initial neutralizing antibody titer (1966–67 surveillance year, 189 students)*

Initial neutralizing antibody titer†	Students				
	No.	Neutralizing antibody seroconversion		CF antibody seroconversion	
		No.	%	*No.*	%
<4	20	5	25	4	20
4	56	15	27	25	45
8	53	9	17	15	28
16	44	4	9	16	36
≥32	16	0	0	6	38

* CF = <1:4 → ≥1:4; neutralization ≥4-fold.
† Reciprocal, initial serum dilution.

level of neutralizing antibody in the November serum ($p = 0.05$), while CF seroconversion occurred with similar frequency irrespective of neutralizing antibody in the November serum.

Strictly comparable data were not available for other years of the study when neutralization tests were only done on sera of students with CF titer rises. However, among those students with CF seroconversion, a significant ($p < .01$) inverse relation between initial neutralizing antibody titer and frequency of significant rise of neutralizing antibody was again seen (table 7).

CLINICAL MANIFESTATIONS OF 229E VIRUS INFECTION

Since students were sampled by culture and serology during periods of good health as well as periods of illness, a controlled estimate could be made of the relation of 229E infection to acute respiratory illness. Briefly, infection, whether determined by virus isolation or CF antibody seroconversion, showed a significant relationship to acute respiratory illness.

Of 12 virus isolations made in the 1961–1962 and 1966–1967 seasons, 11 were from 521 cultures taken during respiratory illness while one came from 1,927 cultures taken during times of good health ($p < .001$).

The multiple serum specimens from the 133 students with CF seroconversions spanned a total of 909 time periods of known health status that could be divided, according to the presence or absence of acute respiratory illness, into "illness periods" and "wellness periods." CF seroconversion to 229E occurred with a significantly greater frequency during "illness periods" than during "wellness periods" (75 seroconversions/245 "illness periods" vs 58/664 "wellness periods" ($p < .001$)).

As cited above, CF seroconversion rates to 229E were not related to presence or absence of homologous neutralizing antibody in sera obtained prior to the time of the CF antibody titer rise. It was also found that the level of neutralizing antibody did not clearly influence the likelihood that illness would accompany CF seroconversion. There was illness during the period of CF seroconversion in 67 per cent of 49 students with initial neutralizing antibody titers <1:8 as compared to 50 per cent of 84 students with initial neutralizing antibody titers ≥1:8 ($p > .05 < .10$). Also, CF seroconversions were illness associated with equal frequency among students with and without concomitant neutralizing antibody titer rises: 60 per cent vs 52 per cent ($p > .3 < .5$).

The clinical characteristics of these respiratory illnesses were not distinctive. Symptoms reported by students during illnesses

TABLE 7

Neutralizing antibody seroconversion to 229E according to pre-rise neutralizing antibody titer: 67 students with CF antibody seroconversions† (Sept. 1961–Sept. 1966)*

Initial neutralizing antibody titer‡	Students		
	No.	Neutralizing antibody titer rise	
		No.	%
<8	19	12	63
8	26	19	73
16	15	5	33
32	7	0	0

* ≥4-fold rise in titer.
† <1:4 → ≥1:4.
‡ Reciprocal, initial serum dilution.

TABLE 8

Seasonal pattern of 229E infections

Surveillance year	No. of students with CF antibody rise* and/or virus isolation†											
	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	July	Aug.
1961–62					(2)†	11, (5)	13, (1)	7	3			
1962–63					2	2		2				
1963–64					5	7	1	6	2			
1964–65								1				
1965–66		1	1		1		1	2	1			
1966–67					7	11	18, (3)	24, (1)	2	2		

* $<1{:}4 \rightarrow \geq 1{:}4$.

† Virus isolations in parentheses.

associated with CF antibody titer rises to this virus were those of undifferentiated acute respiratory infections and did not differ significantly from those reported by these and other students during respiratory infections caused by rhino, RS or parainfluenza viruses.

SEASONAL PATTERN OF 229E INFECTIONS

The seasonal pattern of 229E infections, as evidenced by virus isolation and CF antibody titer rises, is presented in table 8. In each of the six seasons of surveillance, virus isolations and CF seroconversions occurred almost exclusively in the winter and spring months, the exception being 1966–1967 when CF rises were seen in October, November and June. If allowance is made for the time interval by which positive serum specimens post dated the time of infection (two weeks with "illness" specimens and longer with "routine" specimens) most infections occurred during the months of December through April.

DISCUSSION

Certain conclusions seem clear from the observations based on virus isolation and neutralizing antibody seroconversion. Infection with 229E virus was far from uncommon and significantly related to acute respiratory illness. Infection occurred in a winter-spring seasonal pattern similar to that reported previously (9, 11) while the pattern of prevalence over successive years suggested this agent might circulate annually in urban populations with seasons of accentuated prevalence at two- to three-year intervals. Infection was associated with acute respiratory illness clinically indistinguishable from that caused by other common respiratory viruses, and serologically followed by persistently elevated neutralizing and transiently elevated CF antibody titers. Reinfection with 229E appeared to be commonplace and pre-infection neutralizing antibody did not diminish (or increase) the frequency of illness with infection.

Other interpretations of the data must remain tentative to the extent that the specificity of the CF test is not yet clearly defined. Present information concerning this may be summarized as follows. A serologic relation of 229E to viruses of other common groups has not been found in previous or the present studies (1, 7). However, within the coronavirus group, neither the number of human respiratory immunotypes nor the extent of their serologic cross-reactivity are fully known. An antigenic relationship has been demonstrated by CF and HI tests between certain members such as OC 38/43 and MHV, and other less well defined CF cross-reactions may exist within the group (7, 17). However, a common group antigen such as is found in the adeno and influenza viruses has not been demonstrated. In the case of 229E, studies with hyperimmune an-

imal sera have revealed no clear evidence of CF cross-reactions between this virus and MHV, IBV and OC 38/43 (7, 17). Furthermore, sera of human subjects with presumed or known infections with other respiratory coronaviruses have only occasionally shown heterotypic responses to 229E (7, 11, 17). However, even though these studies have not demonstrated extensive CF cross-relations between 229E and presently recognized coronaviruses, the observations are relatively few in number, do reveal some degree of cross-reactivity and do not preclude the possibility that this may exist to an even greater extent with other as yet unrecognized coronaviruses.

Seroconversion to 229E was found more commonly by the CF than the neutralizing antibody test. This was clearly seen in 1966–1967 when both tests were used to follow all students: CF seroconversion occurred in virtually all students with neutralizing seroconversion (29 of 33); and an additional 37 students showed seroconversion by CF test only. In other years of the study when neutralizing antibody tests were only done on students with CF seroconversion, only half of these showed significant rises of neutralizing antibody titer. If it is assumed that in these other years, as in 1966–1967, CF seroconversion identified most of the students with neutralizing antibody titer rises, it was the overall experience that seroconversion to 229E occurred twice as often by the CF as by the neutralizing antibody test.

This difference in CF and neutralizing antibody seroconversion rates could not be entirely accounted for by the decision that 2× CF rises were significant. Although students with 2× CF titer rises showed concomitant neutralizing antibody seroconversion less frequently than those with ⩾4× CF titer rises, 2× reactors appeared to relate to ⩾4× reactors as the lower end of a continuum in which the frequency of neutralizing antibody seroconversion was directly related to the extent of CF antibody titer rise. This was true in each of the high prevalence seasons, and in the overall experience the portion of CF reactors with concomitant neutralizing antibody rises was 35, 45 and 78 per cent of students with 2×, 4× and ⩾8× CF rises, respectively. It should also be noted that 2× and ⩾4× rises were similar in respect to seasonal distribution and the relation of seroconversion to illness.

Thus, either (or both) lesser specificity or greater sensitivity of the CF test also contributed to the difference in CF and neutralizing antibody seroconversion rates. The observations made in these studies do not provide the basis for a clear choice between these alternatives. Infection with other virus(es) cross-reacting with 229E by CF at low titer could be postulated as the origin of the small number of exclusively 2× CF rises that were seen in low frequency seasons, but this would not account for the observation that three of 12 students with CF rises under these circumstances had concomitant neutralizing antibody titer rises to 229E. A similar explanation could be proposed for the finding that pre-existing neutralizing antibody was associated with a diminished frequency of seroconversion by the neutralization test but did not influence the frequency of seroconversion by the CF test. However, if the CF seroconversions in students with high levels of 229E neutralizing antibody in their pre-infection serum were due to infection with one or more other viruses having CF cross-reactivity with 229E, it must be further assumed that something acted to restrict infection rates with the same agents in students with low levels of 229E neutralizing antibody. Otherwise, it might be expected that the latter group of students, subject to infection with 229E as well as the hypothesized cross-reacting agents, would have shown higher CF seroconversion rates than the students subject to infection only by the cross-reacting viruses. The CF seroconversion rates observed were, if anything, lower in students with low levels of pre-infection neutralizing antibody. That different agents would show

such nicely reciprocal infection rates seems somewhat improbable.

It is, however, also possible that the CF test is almost or indeed as specific as the neutralizing antibody test and, where serum specimens are closely spaced, considerably more sensitive. Immunologic factors related to the transient elevation of CF antibody and persistent elevation of neutralizing antibody following infection could play a role in this. The adverse effect of high levels of pre-infection antibody on the serodiagnosis of streptococcal and poliovirus infection has previously been commented upon (18–20). In the case of 229E, long persisting neutralizing antibody may diminish the likelihood that the antigenic stimulus of reinfection will evoke a measureable rise in titer of this antibody while this would not be the case with the CF antibody response. Indeed, if it is assumed that at least a portion of the antibody reacting in the CF test is produced by a segment of the immune system that can develop persisting sensitization, past infection would actually serve to enhance the frequency of measureable CF response to reinfection.

There is not yet sufficient information about the human respiratory coronaviruses to determine the extent to which sensitivity, specificity or both factors account for the observed differences in neutralizing and CF antibody seroconversion rates. Even so certain conclusions can be drawn from the results of the CF survey about the overall role of these viruses in acute respiratory illnesses of humans. Whether in response to infection with only 229E or infection with one or more other serotypes of coronaviruses, CF antibody titer rises to 229E occurred in all six seasons of these studies and were significantly related to acute respiratory illnesses.

If the twofold greater CF than neutralizing antibody seroconversion rate is to be attributable to lesser specificity of the CF test, then among the human respiratory coronaviruses yet to be discovered there must be one or more that circulate widely and share CF antigens with 229E. However, if the difference in seroconversion rates is to be attributed to the factor of sensitivity, several other interesting considerations follow. First, in respect to reinfection, the nearly uniform rate of CF antibody seroconversion to 229E observed among individuals with high as well as low levels of pre-infection neutralizing antibody describes a dissociation between naturally acquired serum antibody and resistance to reinfection (under conditions of natural challenge) that is unusual. Although it has been shown that naturally acquired local respiratory tract antibody is a better predictor than serum antibody, of resistance to reinfection with parainfluenza (21, 22) and RS (23) viruses, a correlation has nevertheless been demonstrable between naturally acquired serum antibody and resistance to reinfection with influenza (24), parainfluenza (21, 22, 25) and rhinoviruses (26). While the apparent dissociation of prechallenge neutralizing antibody and reinfection with 229E as measured by CF antibody seroconversion may simply be an extreme example of the fact that circulating antibody is only an indirect indicator of surface immunity, it could also be interpreted as suggesting that resistance to reinfection is particularly evanescent in the case of the human respiratory coronavirus. That many of the 229E infections were indeed reinfections was clear from both the virus isolation and the neutralizing antibody seroconversion data. Similar findings in respect to OC 43 have been described by Kaye et al. (12) who found that almost 50 per cent of children aged 10–14 years who developed HI seroconversions to OC 43 had pre-existing antibody titers of 1:10 or greater. It might also be emphasized here that not only seroconversion rates but also the frequency with which such seroconversions were illness associated were unaffected by the presence of pre-infection antibody in both the

present studies of 229E and those of OC 43 (12).

An unusual aspect of the seroepidemiology of 229E, referred to above (11), might also be explained on the basis of transience of CF antibody and the enhancement of this antibody response after reinfection. This is the failure to find CF antibody in the acute or convalescent serum specimens of either children hospitalized with lower respiratory tract illness, or those hospitalized for nonrespiratory tract disease, although such antibody was found in sera collected from adults with acute respiratory illness from the same general (but different specific) locale and time period. This could reflect the fact that 229E infections of young children are unusual, or at least seldom of severity that requires hospitalization. However, the experience with other viruses that cause acute respiratory disease in the general population such as influenza, parainfluenza, rhino and RS viruses is that they circulate among children as well as or to a greater extent than among adults (27, 28). Suggesting that this may indeed also be true of 229E virus is the finding by Bradburne (8) of neutralizing antibody to this virus in the sera of eight of 32 children aged 0–5 years and serologic studies in this laboratory indicating a similar prevalence of 229E in the pre-school age group (29). Therefore, lessened serologic responsiveness rather than lower infection rates in this age group must be entertained as a possible explanation of the paucity of CF antibody to 229E observed in the sera of infants and young children.

Finally, this possible enhancement of CF and diminution of neutralizing antibody responsiveness with reinfection could have an important bearing on the procedure of choice for serologic diagnosis of 229E infection in subjects of different ages. Thus, the neutralization test would be the procedure of choice for infants and young children, and the CF test the most sensitive procedure (provided serum specimens are closely spaced) for older children and adults.

References

1. Hamre D, Procknow JJ: A new virus isolated from the human respiratory tract. Proc Soc Exp Biol Med 121: 190–193, 1966
2. Tyrrell DAJ, Bynoe ML: Cultivation of a novel type of common cold virus in organ cultures. Br Med J 1: 1467–1470, 1965
3. McIntosh K, Dees JH, Becker WB, et al: Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. Proc Nat Acad Sci USA 57: 933–940, 1967
4. Almeida JD, Tyrrell DAJ: The morphology of three previously uncharacterized human respiratory viruses that grow in organ culture. J Gen Virol 1: 175–178, 1967
5. Becker WB, McIntosh K, Dees JH, et al: Morphogenesis of avian infectious bronchitis virus and a related human virus (strain 229E). J Virol 1: 1019–1027, 1967
6. Almeida JD, Berry DM, Cunningham CH, et al: Coronaviruses. Nature 220: 650, 1968
7. McIntosh K, Kapikian AZ, Hardison KA, et al: Antigenic relationships among the coronavirus of man and between human and animal coronaviruses. J Immunol 102:1109–1118, 1969
8. Bradburne AF, Bynoe ML, Tyrrell DAJ: Effects of a "new" human respiratory virus in volunteers. Br Med J 3: 767–769, 1967
9. Kapikian AZ, James Jr HD, Kelly SJ, et al: Isolation from man of "avian infectious bronchitis virus-like" viruses (coronaviruses) similar to 229E virus, with some epidemiological observations. J Infect Dis 119: 282–290, 1969
10. Cavallaro JJ, Monto AS: Community-wide outbreak of infection with a 229E-like coronavirus in Tecumseh, Michigan. J Infect Dis 122: 272–279, 1970
11. McIntosh K, Kapikian AZ, Turner HC, et al: Seroepidemiologic studies of coronavirus infection in adults and children. Am J Epidemiol 91: 585–592, 1970
12. Kaye HS, Marsh HB, Dowdle WR: Seroepidemiologic survey of coronavirus (strain OC 43) related infections in a children's population. Am J Epidemiol 94: 43–49, 1971
13. Connelly AP Jr, Hamre D: Virologic studies on acute respiratory disease in young adults. II. Characteristics and serologic studies of three new rhinoviruses. J Lab Clin Med 63: 30, 1964
14. Hamre D, Connelly AP Jr, Procknow JJ: Virologic studies of acute respiratory disease in young adults. III. Some biologic and serologic

characteristics of seventeen rhinovirus serotypes isolated October 1960 to June 1961. J Lab Clin Med 64: 450–460, 1964
15. Sever JL: Application of a microtechnique to viral serological investigations. J Immunol 88: 320–329, 1962
16. Kaye HS, Dowdle WR: Some characteristics of hemagglutination of certain strains of "IBV-like" virus. J Infect Dis 120: 576–581, 1969
17. Bradburne AF: Antigenic relationships amongst coronaviruses. Arch Ges Virusforsch 31: 352–364, 1970
18. Rantz LA: Group A hemolytic streptococcus antibodies. III. A study of the simultaneous infection of a large number of men by a single type. Arch Intern Med 73: 238–240, 1944
19. Gelfand HM, LeBlanc DR, Fox JP, et al: Studies on the development of natural immunity to poliomyelitis in Louisiana. Am J Hyg 65: 367–385, 1957
20. Kaplan EL, Top Jr FH, Dudding BA, et al: Diagnosis of streptococcal pharyngitis: Differentiation of active infection from the carrier state in the symptomatic child. J Infect Dis 123: 490–501, 1971
21. Smith CB, Purcell RH, Bellanti JA, et al: Protective effect of antibody to parainfluenza type I virus. N Engl J Med 275: 1145, 1966
22. Tremonti LP, Lin JL, Jackson GG: Neutralizing activity in nasal secretions and serum in resistance of volunteers to parainfluenza virus type 2. J Immunol 101: 572–577, 1968
23. Mills J, Vankirk JE, Wright PF, et al: Experimental respiratory syncytial virus infection of adults. J Immunol 107: 123–130, 1971
24. Salk JE, Menhe Jr WJ, Francis Jr T: A clinical, epidemiological and immunological evaluation of vaccination against epidemic influenza. Am J Hyg 42: 57–93, 1945
25. Chanock RM, Bell JA, Parrott RH: Natural history of parainfluenza infection. *In* Perspectives in Virology. Chap 11, edited by M Pollard. Burgess Publishing Co, 1961, p 126
26. Cate TR, Rossen RD, Douglas Jr RG, et al: The role of nasal secretion and serum antibody in the rhinovirus common cold. Am J Epidemiol 84: 352–363, 1966
27. Beem MO: Acute respiratory illness in nursery school children: A longitudinal study of the occurrence of illness and respiratory viruses. Am J Epidemiol 90: 30–44, 1969
28. Glezen WP, Loda FA, Clyde Jr WA, et al: Epidemiologic patterns of acute lower respiratory disease of children in a pediatric group practice. J Pediatr 78: 397–406, 1971
29. Beem MO, unpublished data